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Prostaglandin-endoperoxide synthase 2 (*PTGS2*) gene polymorphisms and risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China

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There is evidence that chronic inflammation predisposes to biliary tract cancer and that use of non-steroidal anti-inflammatory drugs (NSAIDs) is protective. Although the mechanisms by which NSAIDs lower cancer risk remain unclear, NSAIDs reduce prostaglandin production by blocking prostaglandin-endoperoxide synthase 2 (PTGS2, commonly known as COX-2), an enzyme induced by pro-inflammatory stimuli that is often overexpressed in malignant tissue. Since variants in the *PTGS2* gene may modify the expression or function of its encoded enzyme to modulate the inflammatory response in the biliary tract, we examined the associations of eight *PTGS2* polymorphisms (−645C→T; Ex3 −8G→C; IVS5 −275T→G; IVS7 +111T→C; Ex10 +127T→C; Ex10 +686 −→ ATTAT→TTATA; Ex10 +837T→C; Ex10 −90C→T) with biliary tract cancer and stones in a population-based case–control study conducted in Shanghai, China. Genotyping was performed for 411 patients with biliary tract cancer (237 gallbladder, 127 extrahepatic bile duct and 47 ampulla of Vater), 895 patients with biliary stones (673 gallbladder, 222 bile duct), and 786 healthy individuals randomly selected from the population. Significant associations were seen only between the Ex10 +837T→C marker and bile duct cancer risk. Relative to individuals with the TT genotype, those carrying the C allele (TC or CC genotype) had a 1.8-fold (95% confidence interval: 1.2–2.7) risk of bile duct cancer. Inferred haplotypes including this risk-conferring allele were also associated with increased bile duct cancer risk of similar magnitude. Our results suggest that a common *PTGS2* variant increases bile duct cancer risk. Further investigation is needed to confirm and extend our findings in studies of biliary tract cancer that more comprehensively examine *PTGS2* and other inflammation-related genes.

Abbreviations: COX, cyclooxygenase; LD, linkage disequilibrium; NSAIDs, non-steroidal anti-inflammatory drugs; PTGS, prostaglandin-endoperoxide synthase; SNP, single nucleotide polymorphism.

Introduction

Cancer of the biliary tract, which includes the gallbladder, extrahepatic bile ducts and ampulla of Vater, is a rare malignancy with poor prognosis for survival (1). Apart from strong links with gallstones and primary sclerosing cholangitis, little is known about its etiology, but these associations are consistent with the notion that the causal pathway involves an inflammatory process of the biliary epithelium (1,2). In a limited number of studies, regular use of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, has also been shown to decrease risk of gallbladder cancer (3,4). In our population-based study of biliary tract cancer in Shanghai, where the incidence of this malignancy has risen rapidly (5), we found a significant 63% reduction in risk for gallbladder cancer and non-significant reductions in risk for bile duct and ampullary cancer with regular aspirin use (6).

Although the exact mechanisms by which NSAIDs lower cancer risk remain unclear, it has been suggested that NSAIDs act by reducing prostaglandin production through inhibition of the enzyme prostaglandin-endoperoxide synthase [PTGS, also commonly called cyclooxygenase (COX)]. In particular, their interaction with the PTGS2 isoform, which is induced by proinflammatory and mitogenic factors (7), appears important, given that PTGS2 overexpression has been observed in neoplastic tissue from multiple sites, including the biliary tree (8–11). Elevated levels of prostaglandins have also been reported to enhance tumor growth (12).

Accordingly, it has been hypothesized that polymorphic variants in the *PTGS2* gene may affect cancer susceptibility by altering its encoded enzyme, either through expression or function, to modulate the inflammatory process. The human *PTGS2* gene, composed of 9 introns and 10 exons, spans 8.3 kb in length on chromosome 1 (1q25.2–25.3) (13). To date, more than 100 *PTGS2* polymorphisms have been identified, but data on their functional significance remain sparse. Of the less than 20 *PTGS2* variants studied in connection with human cancers, those that have been linked to certain cancers, such as the colon (14,15), lung (16–18), prostate (19) and bladder (20), lie primarily in the promoter region or 3′-untranslated region (3′-UTR) of the gene. No studies have examined the relationship between *PTGS2* polymorphisms and risk of biliary cancer or stones. In this report, we extend our investigation of risk factors for biliary disease in Shanghai, assessing the role of

PTGS2 variants in affecting susceptibility to cancer and stones in the biliary tract.

Materials and methods

Study population

Our population-based case–control study of biliary tract cancer included a total of 2092 permanent residents of urban Shanghai, ages 35–74 years: 627 biliary tract cancer case patients (368 gallbladder, 191 bile duct and 68 ampulla of Vater), 1037 biliary stone case patients (774 gallbladder and 263 bile duct) and 959 control subjects (6). Cancer cases were patients newly diagnosed with biliary tract cancer between June 1997 and May 2001, who were identified through a rapid reporting system established between the Shanghai Cancer Institute (SCI) and 42 collaborating hospitals in urban Shanghai. Case ascertainment was >95%. Cancer diagnosis was confirmed for all cases by expert review of histology slides and clinical data from computed tomography (CT) scan, magnetic resonance imaging (MRI), abdominal ultrasound, or endoscopic retrograde cholangiopancreatography (ERCP). Biliary stone cases were patients with biliary stones who were selected by frequency matching to cancer case patients on age (5-year intervals), gender and hospital. Control subjects were adults without a history of cancer randomly selected from all permanent residents listed in the Shanghai Resident Registry, also by frequency matching to cancer cases on age (5-year intervals) and gender. Of the eligible cancer case patients and control subjects, 95% and 82% agreed to participate in the study, respectively. The study protocol was approved by the Institutional Review Boards of the US National Cancer Institute (NCI) and the SCI. All subjects provided written informed consent.

Data and specimen collection

At enrollment, data on epidemiologic factors were collected by in-person interview. To ensure interviews were conducted uniformly among subjects and high quality data were collected, all interviews were tape recorded and reviewed. In addition, 5% of the study subjects were re-interviewed within three months of the original interview, and the concordance of responses to key questions between interviews was >90%. Subjects were also asked to donate an overnight fasting blood sample; half were randomly selected and asked to provide a 24-h urine sample. Gallstone, bile and tissue samples were collected from patients who underwent surgical resection of the biliary tract.

Status for biliary stones, either in the gallbladder or extrahepatic bile ducts, was determined for cancer and stone case patients using data from clinical diagnostic work (abdominal ultrasound, CT scan, ERCP and MRI), medical record review, and interview. For control subjects, status for biliary stones was determined based on interview and abdominal ultrasound data. About 85% of the controls agreed to ultrasound screening for the detection of stones. Individuals who reported having prior cholecystectomy or history of gallstones were classified as having had biliary stones.

Blood processing and genotyping

At an SCI laboratory, blood samples were received, processed and separated within 4 h of collection. Buffy coat samples were stored at −70°C before being shipped to the U.S. on dry ice for DNA extraction by the phenol–chloroform method. The extracted DNA was of good quality, with a high molecular weight and no contamination. For the present study, genomic DNA was available from those who consented to provide blood, and the genotyping of 411 biliary tract cancer case patients (237 gallbladder, 127 bile duct and 47 ampulla of Vater), 895 biliary stone case patients (673 gallbladder and 222 bile duct), and 786 control subjects was carried out at the NCI Core Genotyping Facility (CGF; Gaithersburg, MD). Laboratory personnel were blinded to the case–control status of the samples.

Each *PTGS2* polymorphism was selected *a priori* based on two criteria: (i) having either a reported prevalence of at least 5% for the variant allele among Asians as reported in the SNP500Cancer database (21) or published evidence of an association with disease (particularly cancer) and (ii) availability of a validated assay at the CGF. Genotyping methods by TaqMan assay for the eight *PTGS2* polymorphisms selected (−645C→T rs20420, Ex3 −8G→C rs5277, IVS5 −275T→G rs20432, IVS7 +111T→C rs4648276, Ex10 +127T→C rs5273, Ex10 +686 −→ATTAT→TTATA rs4648291, Ex10 +837T→C rs5275, Ex10 −90C→T rs689470) can be referenced online at http://snp500cancer.nci.nih.gov. The genotyping completion rate for each assay exceeded 98%.

To evaluate the quality of genotyping, 80 internal blind duplicates from four individuals spaced at varying intervals between study samples were genotyped. Among the duplicate samples, >99% concordance for the genotyping of each *PTGS2* marker was achieved.

Statistical analysis

Single marker associations. Among control subjects, genotype frequencies for each *PTGS2* marker were examined for deviation from Hardy–Weinberg equilibrium (HWE) using the χ^2-test. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) to assess the relationship of each *PTGS2* polymorphism with biliary tract cancer risk at each anatomic subsite, adjusting for age and gender. Gallbladder cancer case patients were compared with control subjects without a history of cholecystectomy, while bile duct and ampullary cancer case patients were compared with all control subjects. For each *PTGS2* marker, the homozygous genotype of the more frequent allele was used as the referent category. Additional logistic regression models were run with further adjustment for biliary stone status to evaluate potential confounding by this factor, since individuals diagnosed with biliary tract cancer and stones may have similar genetic susceptibility profiles. The risk of biliary stones associated with each *PTGS2* polymorphism was also estimated, controlling for age and gender, by comparing gallbladder or bile duct stone cases to the subset of population controls without stones. Tests for linear trend in risk according to the number of copies of the variant allele (0, 1 or 2) were conducted to assess potential dose–response effects (22). To account for multiple comparisons, a summary *P*-value of the overall association between polymorphisms in the *PTGS2* gene and each outcome of interest was derived using the Simes global test (23), which controls the familywise error rate (i.e. the chance that any *PTGS2* polymorphism is erroneously declared to be associated with disease will be ≤5%, if in fact no *PTGS2* polymorphism is truly associated) (24).

Haplotype associations. Among population controls, the presence of linkage disequilibrium (LD) between loci exhibiting genetic variation was assessed by calculating pairwise Lewontin's D′ and r^2 values using Haploview version 3.11 (25). Using Haplostats (26) in R version 2.0.1, we estimated haplotype frequencies for case patients and control subjects separately, taking into account the extent of haplotype uncertainty, according to the expectation-maximization (EM) algorithm (27). Following similar procedures as outlined above for single markers, we performed the haplotype analysis assuming an additive model, using the most common haplotype as the referent category. Associations between the *PTGS2* haplotypes with observed frequencies >1% and biliary tract cancer and stones were evaluated.

Results

Population characteristics

In this study, there were more women than men diagnosed with gallbladder cancer or biliary stones, but more men than women with cancers of the bile duct or ampulla of Vater (Table I). Although the age distribution was similar between cancer cases and controls, biliary stone cases were slightly younger. Those with cancer, particularly of the gallbladder, were more likely to have had biliary stones in comparison to population controls. Also relative to controls, cases with either gallbladder cancer or biliary stones were more likely to have a higher body mass index. Use of cigarettes and alcohol was most common among cases with bile duct cancer, but least common among cases with gallstones. Aspirin use was extremely low; 6.4% of the control subjects took aspirin regularly.

Variation was observed for five of the eight *PTGS2* polymorphisms studied: Ex3 −8G→C, IVS5 −275T→G, IVS7 +111T→C, Ex10 +837T→C, and Ex10 −90C→T. Among population controls, minor allele frequencies for these markers ranged from 1 to 17%, and the genotype frequencies of each marker showed no deviation from HWE ($P \geq 0.01$). None of the cancer cases carried the homozygous variant genotype for the Ex3 −8, IVS5 −275, IVS7 +111, and Ex10 −90 loci, although a few stone cases and controls did.

Single marker associations

Of the five *PTGS2* markers showing genetic variation, significant associations were found only for Ex10 +837T→C, with increased risks for cancer of the bile duct and ampulla of Vater and for bile duct stones (Table II). For this marker, 69.5, 27.8

Table I. Distribution of selected characteristics by case–control status

Characteristic, *n* (%)	Control subjects		Cancer case patients			Biliary stone case patients	
	All ($n = 786$)	No biliary stones ($n = 592$)	Gallbladder ($n = 237$)	Bile duct ($n = 127$)	Ampulla of vater ($n = 47$)	Gallbladder ($n = 673$)	Bile duct ($n = 222$)
Gender							
Male	305 (38.8)	252 (42.6)	65 (27.4)[a]	76 (59.8)[b]	24 (51.1)	224 (33.3)[c]	105 (47.3)
Female	481 (61.2)	340 (57.4)	172 (72.6)	51 (40.2)	23 (48.9)	449 (66.7)	117 (52.7)
Age at interview, years							
<55	107 (13.6)	97 (16.4)	32 (13.5)	18 (14.2)	4 (8.5)	216 (32.1)[c]	53 (23.9)[c]
55–64	224 (28.5)	169 (28.5)	62 (26.2)	32 (25.2)	9 (19.2)	186 (27.6)	66 (29.7)
≥65	455 (57.9)	326 (55.1)	143 (60.3)	77 (60.6)	34 (72.3)	271 (40.3)	103 (46.4)
Biliary stones	194 (24.7)	0 (0.0)	146 (61.9)[a]	86 (67.7)[b]	28 (59.6)[b]	673 (100)[c]	222 (100)[c]
Body mass index[d], kg/m^2							
<23	391 (49.8)	323 (54.7)	90 (38.1)[a]	63 (49.6)	21 (44.7)	254 (37.8)[c]	89 (40.1)[c]
23–24	166 (21.2)	123 (20.8)	47 (19.9)	33 (26.0)	11 (23.4)	164 (24.4)	51 (23.0)
≥25	228 (29.0)	145 (24.5)	99 (42.0)	31 (24.4)	15 (31.9)	254 (37.8)	82 (36.9)
Cigarette use[e]	237 (30.2)	187 (31.6)	64 (27.1)	56 (44.1)[b]	20 (42.6)	161 (23.9)[c]	80 (36.0)
Alcohol use[f]	162 (20.6)	134 (22.6)	36 (15.2)	42 (33.1)[b]	12 (25.5)	101 (15.0)[c]	41 (18.6)
Aspirin use[g]	50 (6.4)	35 (5.9)	8 (3.4)	4 (3.2)	1 (2.1)	37 (5.5)	4 (1.8)[c]

[a]Compared with population controls without prior cholecystectomy, $P < 0.05$.
[b]Compared with population controls, $P < 0.05$.
[c]Compared with population controls without biliary stones, $P < 0.05$.
[d]Calculated using self-reported weight and height at 5 years prior to interview in units of kg/m^2.
[e]At least one per day for six months or longer.
[f]At least once a week for six months or longer.
[g]At least twice a week for longer than a month one year prior to interview.

and 2.7% of the controls carried the TT, TC and CC genotype, respectively. Carriers of the Ex10 +837 C allele (TC and CC genotypes combined) had a 1.8-fold (95% CI: 1.23–2.67) risk of bile duct cancer ($P = 0.003$), relative to those having the TT genotype. This excess risk was observed among the heterozygous (OR = 2.01, 95% CI: 1.20–2.67) and not the homozygous carriers (OR = 0.99, 95% CI: 0.72–5.60), but only 4% ($n = 5$) of the bile duct cancer cases were homozygous carriers of the Ex10 +837 C allele in this study. After adjustment for biliary stones, the association between the Ex10 +837 C allele and bile duct cancer persisted, with the magnitude slightly attenuated (OR = 1.69, 95% CI: 1.11–2.56). The CC genotype, but not the C allele, of the *PTGS2* Ex10 +837 marker was associated with 3-fold risks of ampullary cancer and bile duct stones. After accounting for multiple comparisons, however, only the association between *PTGS2* and bile duct cancer remained statistically significant ($P = 0.02$).

Haplotype associations

Among control subjects, strong LD was present among four of the five markers showing genetic variation, with pairwise D′ values ranging from 0.9 to 1.0. Haplotype analysis on the four closely-linked loci or all five loci yielded similar results, since no allelic variation occurred at Ex10 −90 among the major haplotypes. Therefore, only results for those haplotypes inferred from the four loci, Ex3 −8G→C, IVS5 −275T→G, IVS7 +111T→C, and Ex10 +837T→C, are presented in Table III. Based on these four loci, we inferred four major *PTGS2* haplotypes, specifically GTTT, GTTC, GGCC and CTTT, with corresponding frequencies of 79.5, 11.2, 4.8 and 3.7% among control subjects.

Consistent with our single marker results, haplotype-specific associations were limited to bile duct cancer. Relative to the most frequent haplotype GTTT, the two haplotypes including the Ex10 +837 C allele were associated with increased risk of bile duct cancer (GTTC, OR = 1.66 per copy, 95% CI: 1.13–2.46; GGCC, OR = 1.75 per copy, 95% CI: 0.99–3.08). The association of the GTTC haplotype persisted (OR = 1.60 per copy, 95% CI: 1.06–2.44), after further adjustment for biliary stones.

Discussion

In this population-based study of biliary tract cancer in Shanghai, China, we found that the *PTGS2* Ex10 +837 C allele was associated with a 1.8-fold risk of bile duct cancer. Similar increases in bile duct cancer risk were also associated with the two common haplotypes that included this risk-conferring allele, suggesting that the observed haplotype associations reflect the influence of this single polymorphic marker. No clear effects of the other *PTGS2* polymorphisms on risk of biliary tract cancer or biliary stones were found.

Although this is the first study to assess whether variants in the *PTGS2* gene are related to risk of biliary tract cancer, our findings are biologically plausible. Inflammatory conditions of the bile duct, particularly primary sclerosing cholangitis, are strongly associated with bile duct cancer (28). Increased PTGS2 expression has been observed in bile duct carcinoma, as well as in bile duct epithelial cells of primary sclerosing cholangitis, relative to normal tissue (11). It is therefore possible that variants in the *PTGS2* gene affect susceptibility to bile duct cancer by modifying the inflammatory response through changes in the expression or function of the PTGS2 enzyme.

In our study, five of the eight *PTGS2* markers showed genetic variation, of which only Ex10 +837T→C was associated with an increased risk of bile duct cancer. Two reasons may account for the relation to this one *PTGS2* marker. First, there was sufficient statistical power to detect a modest increase in risk associated with this single nucleotide polymorphism

Table II. ORs and 95% CIs for biliary tract cancer and biliary stones in relation to selected *PTGS2* polymorphisms

PTGS2 Polymorphism Genotype	Control Subjects		Cancer case patients												Biliary stone case patients							
			Gallbladder				Extrahepatic bile duct				Ampulla of Vater				Gallbladder				Extrahepatic bile duct			
	n	(%)	*n*	(%)	OR*	95% CI	*n*	(%)	OR*	95% CI	*n*	(%)	OR*	95% CI	*n*	(%)	OR*	95% CI	*n*	(%)	OR*	95% CI
Ex3 −8G→C (rs5277)																						
GG	709	92.6	214	92.2	1.00	—	109	89.3	1.00	—	43	93.5	1.00	—	610	93.7	1.00	—	201	93.5	1.00	—
GC	57	7.4	18	7.8	1.07	0.61–1.88	13	10.7	1.44	0.76–2.76	3	6.5	0.81	0.24–2.72	40	6.1	0.86	0.54–1.36	14	6.5	0.95	0.51–1.79
CC	0	0.0	0	0.0			0	0.0			0	0.0			1	0.2			0	0.0		
IVS5 −275T→G (rs20432)																						
TT	696	89.8	210	90.1	1.00	—	106	84.1	1.00	—	40	87.0	1.00	—	602	90.4	1.00	—	193	87.7	1.00	—
TG	75	9.7	23	9.9	1.04	0.63–1.72	20	15.9	1.73	1.01–2.99	6	13.0	1.47	0.60–3.60	60	9.0	1.02	0.68–1.52	25	11.4	1.33	0.80–2.22
GG	4	0.5	0	0.0			0	0.0			0	0.0			4	0.6	2.19	0.39–12.15	2	0.9	3.19	0.44–22.96
TG or GG	79	10.2	23	9.9	1.00	0.61–1.64	20	15.9	1.65	0.96–2.83	6	13.0	1.39	0.57–3.39	64	9.6	1.06	0.71–1.57	27	12.3	1.40	0.85–2.30
IVS7 +111T→C (rs4648276)																						
TT	708	90.7	214	90.3	1.00	—	109	85.8	1.00	—	41	87.2	1.00	—	610	90.9	1.00	—	193	88.1	1.00	—
CT	72	9.2	23	9.7	1.10	0.66–1.82	18	14.2	1.61	0.92–2.84	6	12.8	1.53	0.63–3.77	59	8.8	1.01	0.67–1.51	25	11.4	1.34	0.80–2.24
CC	1	0.1	0	0.0			0	0.0			0	0.0			2	0.3			1	0.5		
CT or CC	73	9.3	23	9.7	1.08	0.65–1.79	18	14.2	1.60	0.91–2.80	6	12.8	1.51	0.62–3.71	61	9.1	1.05	0.70–1.57	26	11.9	1.40	0.84–2.33
Ex10 +837T→C (rs5275)																						
TT	541	69.5	165	69.9	1.00	—	70	55.6	1.00	—	30	66.7	1.00	—	461	69.0	1.00	—	147	66.5	1.00	—
TC	216	27.8	61	25.8	0.95	0.67–1.33	51	40.5	2.01	1.20–2.67	11	24.4	0.95	0.46–1.93	192	28.7	1.02	0.80–1.32	63	28.5	1.06	0.74–1.50
CC	21	2.7	10	4.2	1.58	0.71–3.51	5	4.0	0.99	0.72–5.60	4	8.9	3.51	1.12–11.01	15	2.2	1.05	0.47–2.35	11	5.0	3.09	1.30–7.33
				$P_{trend} = 0.70$				$P_{trend} = 0.004$				$P_{trend} = 0.23$				$P_{trend} = 0.83$				$P_{trend} = 0.10$		
TC or CC	237	30.5	71	30.0	1.00	0.73–1.38	56	44.5	1.81	1.23–2.67	15	33.3	1.18	0.62–2.24	207	30.9	1.03	0.80–1.31	74	33.5	1.17	0.84–1.64
Ex10 −90C→T (rs689470)																						
CC	750	98.4	229	99.1	1.00	—	121	98.4	1.00	—	44	97.8	1.00	—	645	98.9	1.00	—	213	99.1	1.00	—
CT	12	1.6	2	0.9	0.52	0.12–2.40	2	1.6	1.08	0.23–4.99	1	2.2	1.49	0.19–11.91	7	1.1	0.69	0.25–1.93	2	0.9	0.64	0.13–3.00
TT	0	0.0	0	0.0			0	0.0			0	0.0			0	0.0			0	0.0		
P-value for Simes Test					0.80				0.02				0.73				0.82				0.21	

*Adjusted for age and gender.

Table III. ORs and 95% CIs for biliary tract cancer and biliary stones in relation to the common *PTGS2* haplotypes

Haplotype[a]	Control subjects	Cancer case patients									Biliary stone case patients					
		Gallbladder			Bile duct			Ampulla of Vater			Gallbladder			Bile duct		
	(%)	(%)	OR[b]	95% CI	(%)	OR[b]	95% CI	(%)	OR[b]	95% CI	(%)	OR[b]	95% CI	(%)	OR[b]	95% CI
G-T-T-T	79.5	79.0	1.00	—	71.7	1.00	—	76.8	1.00	—	80.4	1.00	—	77.8	1.00	—
G-T-T-C	11.2	11.8	1.06	0.76–1.47	15.1	1.66	1.13–2.46	14.5	1.34	0.72–2.47	11.1	1.00	0.77–1.30	12.2	1.21	0.86–1.69
G-G-C-C	4.8	4.9	1.07	0.65–1.76	7.1	1.75	0.99–3.08	5.3	1.55	0.64–3.75	4.6	1.03	0.69–1.54	6.1	1.46	0.88–2.40
C-T-T-T	3.7	3.9	1.09	0.62–1.93	4.2	1.67	0.86–3.22	2.3	0.87	0.26–2.95	2.7	0.86	0.53–1.41	2.7	1.01	0.54–1.91

[a]Includes the following four *PTGS2* SNPs: Ex3 −8G→C, IVS5 −275T→G, IVS7 +111T→C, and Ex10 837T→C.
[b]Adjusted for age and gender.

(SNP) because its minor allele frequency among controls was 17%, the highest among all eight markers included in the study. For the other *PTGS2* markers with minor allele frequencies ≤5%, the statistical power of detecting modest effects was limited. Second, although little is currently known about the function of most *PTGS2* polymorphisms, the genomic location of the *PTGS2* Ex10 +837T→C SNP suggests that it is functionally important. This SNP lies in a putative functional sequence of the 3′-UTR that regulates mRNA stability and translation (29), which may influence the extent of PTGS2 production and activity and thereby affect cancer risk.

Data on the role of the *PTGS2* Ex10 +837T→C SNP in cancer are emerging, yet remain sparse (14,16–18,30). As in our study, the Ex10 +837 C allele was related to risk of non-small cell lung cancer in a Norwegian population (16), but this finding was not replicated in recent investigations, including a large hospital-based study conducted in six European countries (30) and two population-based studies in China (17) and Denmark (18). In the one study of colorectal cancer (14), no association was found for the Ex10 +837T→C marker, but an increased risk was associated with another SNP further downstream in the 3′-UTR, which we did not genotype. Although it is not known whether these two SNPs are linked, future investigations should assess whether the association observed for bile duct cancer could be explained by other *PTGS2* SNPs occurring in strong LD with the Ex10 +837T→C SNP.

Accumulating data from studies examining the relationship of *PTGS2* polymorphisms to other epithelial cancers also indicate that variations occurring within certain regions of the *PTGS2* gene may be more relevant to cancer risk. Most of the *PTGS2* polymorphisms identified to date have been either intronic or synonymous substitutions in the coding region (31). However, of the few intronic or synonymous *PTGS2* variants examined thus far (14,16), including those in our study, none has been linked to cancer risk. Whereas for variants that lie in the promoter region of the *PTGS2* gene (14–16,19,20,32), most associations have been reported for those that map to transcription factor binding sites. Taken together, these data suggest that, in future studies, polymorphisms in the *PTGS2* promoter and 3′-UTR that influence gene transcription or translation should be closely investigated in relation to cancer risk.

A notable strength of this study was the inclusion of two separate case groups, one for biliary tract cancer and one for biliary stones, as it offered a unique opportunity to assess whether risks associated with various exposures, including genetic susceptibility, are similar between these two closely related conditions. Our results suggest that the association of the *PTGS2* Ex10 +837T→C marker is specific to bile duct cancer. In fact, confounding by biliary stones, if any, should be minimal, since this marker was not associated with the risk of biliary stones, and its association with bile duct cancer persisted after adjustment for biliary stones. Although the possibility of a false positive association cannot be ruled out entirely, we accounted for multiple hypothesis testing in our analysis to reduce the likelihood of reporting erroneous results. Additional strengths of our study included nearly complete case ascertainment for cancer, a high participation rate, and confirmation of case status by comprehensive pathologic and clinical review, which minimized the potential for selection, survival and misclassification bias. The use of rigorous quality control measures in the laboratory further ensured minimal genotype misclassification.

Several study limitations should also be noted. First, like most genetic susceptibility studies, our coverage of the *PTGS2* gene was limited, since SNP selection was not based on complete sequencing data for our target population. Only eight of the more than hundred *PTGS2* polymorphisms identified to date were examined, and given the vast number of *PTGS2* variants, the association with bile duct cancer may reflect the influence of other alleles in LD. Incomplete gene coverage also limited the examination of true *PTGS2* haplotypes. Second, due to low minor allele frequency and the small number of bile duct and ampullary cancer cases, there was limited statistical power to evaluate certain main effects. Third, despite being the largest population-based study of biliary tract cancer to date, the low prevalence (<7%) of inflammation-related exposures that may influence PTGS2 activity (e.g. NSAID use and ulcerative colitis), coupled with minimal allelic variation and the number of cancer case patients, in the study population precluded the assessment of gene–environment interactions. Lastly, our findings have limited generalizability, largely because this study was conducted on a fairly homogeneous Chinese population. Yet for the same reason, it is likely that any influence of population stratification was minimal.

In conclusion, this case–control study showed that a common variant located in the 3′-UTR of the *PTGS2* gene is associated with an increased risk of bile duct cancer, consistent with the view that chronic inflammation is a key impetus to the carcinogenic process in the biliary tree. Further studies of biliary tract cancer are needed that provide a more comprehensive coverage of *PTGS2* and other genes involved in inflammation-related pathways.

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